Practitioner's Docket No. 2260/106

CHAPTER II

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IB99/01553	17 September 1999	18 September 1998

TITLE OF INVENTION
Process for Obtaining HMG-CoA Reductase Inhibitors of High Purity

APPLICANT(S)
Grahek et al.

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date 03 January 2001, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL 543502079 US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Karen A. Buchanan

(type or print name of person mailing paper)

Karen A. Buchanan

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☒*	TOTAL CLAIMS	29 −20=	9	× $18.00=	$ 162.00
	INDEPENDENT CLAIMS	1 −3=	0	× $~~78.00~~=	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $96.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $670.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $690.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $970.00 ☒ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) ..... $~~840.00~~ 860				860.00
	Total of above Calculations				= 1,022.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				–
	Subtotal				1,022.00
	Total National Fee				$ 1,022.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				40.00
TOTAL	Total Fees enclosed				$ 1,062.00

*See attached Preliminary Amendment Reducing the Number of Claims.

i. ☐ A check in the amount of _____ to cover the above fees is enclosed.

ii. ☒ Please charge Account No. 19-4972 in the amount of $ 1,062.00
A duplicate copy of this sheet is enclosed.

***WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☐ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☒ has been transmitted

i. ☒ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308): 30 March 2000

ii. ☐ by applicant on _____.
Date

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____.
Date

d. ☐ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: *The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.*

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.
Date

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.): 23 November 1999

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☒ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☒ Annex(es) to the international preliminary examination report

a. ☒ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☒ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☒ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____. Date

b. ☒ is submitted herewith, and such oath or declaration

i. ☒ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☐ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☒ is transmitted herewith.

b. ☒ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): 30 March 2000

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____. Date

12. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449 (PTO/SB/08A and 08B).

☐ Copies of citations listed.

b. ☒ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____. Date

13. ☒ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☒ FORM PTO 1595 is also attached.

_____

_____

_____

14. ☒ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☒ International Publication No. WO 00/17182

i. ☒ Specification, claims and drawing

ii. ☐ Front page only

c. ☐ Preliminary amendment (37 C.F.R. § 1.121)

d. ☒ Other

Written Opinion

Response to Written Opinion

15. ☒ The above checked items are being transmitted

a. ☒ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 19-4972.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☒ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

Karen A. Buchanan

SIGNATURE OF PRACTITIONER

Reg. No.: 37,790

Karen A. Buchanan

(type or print name of practitioner)

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09/720095

Title of the invention

Process for obtaining HMG-CoA reductase inhibitors of high purity

Technical Field

Lovastatin, pravastatin, simvastatin, mevastatin, atorvastatin and derivatives and analogs thereof are known as HMG-CoA reductase inhibitors and are used as antihypercholesterolemic agents. The majority of them are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus, Monascus, Nocardia, Amycolatopsis, Mucor* or *Penicillium* genus, some are obtained by treating the fermentation products using the method of chemical synthesis or they are the products of total chemical synthesis.

The purity of the active ingredient is an important factor for manufacturing the safe and effective pharmaceutical, especially if the pharmaceutical product must be taken on a longer term basis in the treatment or prevention of high plasma cholesterol. The accumulation of the impurities from the pharmaceuticals of lower purity may cause many side effects during the medical treatment.

The present invention relates to a new industrial process for the isolation of HMG-CoA reductase inhibitors using so-called displacement chromatography. Use of the invention enables to obtain HMG-CoA reductase inhibitors of high purity, with high yields, lower production costs and suitable ecological balance.

Prior Art

The processes for the isolation and purification of antihypercholesterolemic agents disclosed in the earlier patents include a variety of combinations of extraction, chromatography, lactonisation and crystallisation methods. The purity of the final product obtained by these procedures comply with the USP standards but the yields of the desired product are relatively low. In addition, they require both large amounts of organic solvents and the large equipment suited for these quantities.

The isolation process disclosed in WO 92/16276 provides the solution for obtaining HMG-CoA reductase inhibitors of purity greater than 99.5% with the use of industrial HPLC (high performance liquid chromatography) equipment. According to WO 92/16276 the crude HMG-CoA reductase inhibitor, with a purity of ≥ 85%, is dissolved in an organic solvent or in a solution of organic solvent and water. The mixture is then buffered to a pH between 2 and 9 and placed on the HPLC column. After the HMG-CoA reductase inhibitor peak of interest is collected, a portion of solvent is removed and the water is added or alternatively two-thirds of the solvent mixture are removed and the HMG-CoA reductase inhibitor is crystallised. At the end, the purity of the product obtained by this process is at least 99.5% with the yield of around 90%.

The method disclosed in WO 92/16276 enables obtaining of HMG-CoA reductase inhibitors of high purity, with relatively high yields, the disadvantage of the method over the conventional chromatography columns are relatively small quantities of the substance loaded per HPLC column. Small samples to be fed into the column are also related with increased number of repetitions of the isolation operations in order to obtain sufficient quantities of the desired substance, and consequently

large amount of the solvents used resulting in higher production costs.

Displacement chromatography method, the basis of the present invention, does not substantially differ from previously used chromatography methods.

Displacement chromatography is based on competition of the components of the sample fed into the column for active sites on the stationary phase. Individual components of the sample displace one another like a train, the displacer, having the very high affinity for the stationary phase and travelling behind the fed sample along the column, drives the separation of the sample components into one-compartment zones which move at the same velocity as the displacer. Concentrating of individual components is carried out simultaneously with the purification.

The principle of displacement chromatography method is relatively old as it has been known since 1943 but it was introduced into practice as late as 1981 because of the lack of efficient columns (Cs. Horvath et al., J. Chromatogr., 215 (1981) 295; J. Chromatogr., 330 (1985) 1; J. Chromatogr., 440 (1988) 157). These papers, introduced herein by way of reference, describe the analytic and preparative separation and purification of biologically active peptides and polymyxin antibiotics (polypeptides) using reversed-phase high performance liquid chromatography columns in the displacement mode. For polymyxins octadecyl silica gel columns 250 x 4.6 mm, particle size 5µm, 10% acetonitrile in water as the mobile phase and different tetraalkylammonium halogenides as the displacer were used.

In recent investigations in the field of displacement chromatography (S.M. Cramer et al., Enzyme Microb. Technol., 11 (1989) 74; Prep. Chromatogr., 1 (1988) 29; J. Chromatogr., 394 (1987) 305; J. Chromatogr., 439 (1988)

341; J. Chromatogr., 454 (1988) 1 (theoretic optimisation)); A. Felinger et al., J. Chromatogr., 609 (1992) 35 (theoretic optimisation), all papers being introduced herein by way of reference) similar columns were used; the mobile phase was methanol in the phosphate buffer, the displacer was 2-(2-t-butoxyethoxy)ethanol (BEE) in acetonitrile and sodium acetate. Different peptides, proteins and cephalosporin C antibiotic were used as the samples.

US Pat. No. 5,043,432 (27.08.1991) and EP 416.416, respectively, describe the method for purifying certain low molecular (below 1000 daltons) peptides (in particular, tuftsin and synthetic derivatives thereof) with displacement ion-exchange chromatography where the stationary phase used is cationic-exchange resin, the transporter solvent is water or dilute solutions of a variety of strong acids, and the displacer used is triethylenetetraammonuim salt in different concentrations. In US patent application 08/875,422, yet unpublished, the use of displacement chromatography for the isolation and purification of vancomycin is described.

Technical Solution

It is sometimes difficult to obtain the active substance of high purity in a large scale as many technologies applicable to a laboratory scale are not sufficiently economical in large scale production operations to justify use thereof or do not meet the environmental criteria. The above facts compel the industry to search for new technologies that will provide both the high-quality product and the economically and ecologically acceptable production.

The present invention has solved the drawbacks of the processes known from the older patents and other literature as it enables to obtain the pure HMG-CoA

reductase inhibitors and, additionally, the purifying process *per se* is not time-consuming providing high yields, using small amounts of solvents. The process is nature friendly; in addition, it is not demanding in terms of space and energy thus enabling an economical large scale production.

Description of the invention

The present invention provides a process for the purification of HMG-CoA reductase inhibitors employing displacement chromatography. That is, at least one of the steps in the process of the purification of crude HMG-CoA reductase inhibitor includes displacement chromatography.

The HMG-CoA reductase inhibitor to be purified is, for example, selected from the group consisting of mevastatin, pravastatin, lovastatin, simvastatin, fluvastatin and atorvastatin. The selected inhibitor may be in the lactone form or in the form of the acid or the salt thereof for being purified by means of displacement chromatography.

The displacement chromatography being characteristic for the process of the present invention preferably includes the following steps:

a) conditioning a chromatography column with an appropriate mobile phase,

b) feeding the crude HMG-CoA reductase inhibitor dissolved in the mobile phase,

c) introducing the displacer for displacing the HMG-CoA reductase inhibitor from the column, and

d) obtaining the purified HMG-CoA reductase inhibitor.

The purified HMG-CoA reductase inhibitor is preferably obtained by

d1) collecting the fractions and

d2) analyzing the fractions with analytical HPLC and pooling the fractions depending on the quality of purity.

After the purified HMG-CoA reductase inhibitor has been obtained, the chromatography column may be regenerated by washing of the column with alcohol/water mixture to elute the displacer.

HMG-CoA reductase inhibitors obtained in the herein-described manner are then isolated from the mobile phase according to the methods already known from the state of prior art, for example by lyophilisation or, prefarably, by crystallization to obtain the lactone form, the acid form or the salt form (preferably alkaline or earth alkaline salts) thereof.

The fractions containing a considerable percentage of HMG-CoA reductase inhibitors, in addition to impurities, may be re-subjected to the process resulting in the total yield exceeding 95%.

The stationary phase used is a reverse phase where natural (silica gel with alkyl chains of a different length) or synthetic (C-18 or C-8 organic) stationary phases are suitable. Preferably, a synthetic cross-linked polymer matrix of styrene and divinylbenzene is used. The particle size of the stationary phase is suitably from 3 to 20 μm, preferably between 7 and 15 μm.

The mobile phase used is preferably selected from water, acetonitrile/water solution and aqueous solutions of lower (preferably C_1-C_4) alcohols, buffered dilute solutions of organic, halogenated organic or inorganic acids, e.g. formic, acetic, propionic, hydrochloric, boric, phosphoric, carbonic or suphuric acids with cations of alkaline metals, with ammonia or with amines. Water and aqueous solutions with acetonitrile and especially with methanol or ethanol are particularly preferred, and the content of the organic solvent in the aqueous solutions preferably is 80% or below, more preferably 45% or below

and particularly 30% or below. Since toxic methanol in the mobile phase may be replaced by less toxic ethanol, or may be at least partially replaced by water with good results, removal of waste solvents is simpler, therefore, the present invention is a marked improvement compared to the state of prior art judging from the ecological aspect.

The pH of the mobile phase used is preferably between 4.5 and 10.5, more preferably between 6.5 and 8, and particularly around 7. The flow rate of the mobile phase through the column is suitably adjusted to lie between 1.5 and 30 ml/(min cm^2), preferably between 3 and 15 ml/(min cm^2). At the time when the displacer is introduced into the chromatography column by being mixed with the mobile phase, the flow rate is preferably adjusted to lie between 1.5 and 15 ml/(min cm^2) and particularly between 3 and 10 ml/(min cm^2), because higher flow rates cause the dilution of the samples to be collected, and also the separation becomes worse.

The displacer suitably is a compound having an amphiphilic structure, such as surfactants, detergents and the like. Examples of the displacer are long chain alcohols, long chain carboxylic acids, long chain alkyl ammonium salts, aromatic dicarboxylic acid esters, oxo- and dioxo-alcohols, polyalkylene polyglycol ethers such as diethylene glycol mono- (or di-)alkylethers, polyaryl or polyalkylene polyaryl ethers such as Triton® X-100, etc. The aforementioned "long chain" means an alkyl chain having at least a C_4-chain, preferably at least a C_{10}-chain and more preferably at least a C_{14}-chain or longer.

The concentration of the displacer in the mobile phase is suitably adjusted to be from 1 to 35%, preferably from 2 to 20% and particularly from 7 to 14%.

In the preferred embodiment of controlling the quality of purity in the individual fractions eluted from the chromatography column, an analytical HPLC method directed

to the HMG-CoA reductase inhibitors to be analyzed may be carried out as described in the following.

The sample to be analysed is diluted 100 times with the mobile phase containing 20 mM aqueous NH_4HCO_3 solution with acetonitrile (the proportion of acetonitrile is adjusted such that the retention factor of the analyte is between 5 and 10). 10 µl of this sample is placed on Hypersil ODS column (Hypersil, the United Kingdom, particle size 3µm, column size 50 × 4.6 mm) for high performance liquid chromatography. The column is washed with the mobile phase at the flow rate of 2 ml/min. Absorbance is measured at 235 nm. HPLC purity of the sample is calculated from the ratio between the areas of individual peaks in the chromatogram.

After completed chromatography the stationary phase is preferably regenerated, for example using the mobile phase with 20 to 100% aqueous solution of lower alcohol.

The invention is illustrated but in no way limited by the following examples.

EXAMPLES

Example 1

Crude sodium salt of pravastatin (1.0 g, HPLC purity 88%, assay 85%) was dissolved in 10 ml of the mobile phase A (distilled water), pH was adjusted to 7 with 0.2M aqueous NaOH solution and filtered. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 × 10 mm. The column was washed with the mobile phase B containing 7% of diethyleneglycol monobutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 0.5 ml fractions were collected with an initial increase in the

absorbance. When the signal decreased the column was washed with 25 ml of 70% methanol. The obtained fractions were analyzed by the herein above-described HPLC analytical method. The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions (7 ml) the HPLC purity was 99.8%.

Example 2

Crude sodium salt of pravastatin (0.4 g, HPLC purity 88%, assay 85%) was dissolved in 5 ml of the mobile phase A (distilled water), pH was adjusted to 7 with 0.2M aqueous NaOH solution and filtered. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Kromasil 100 C-18 column (EKA Chemicals AB, Sweden), particle size 10 µm, column size 200 × 10 mm. The column was washed with the mobile phase B containing 7% of Triton X-100 in mobile phase A at the flow rate of 1 ml/min. Absorbance was measured at 260 nm, and the 0.5 ml fractions were collected with an initial increase in the absorbance. The obtained fractions were analysed by the above described HPLC analytical method. The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions (3 ml) the HPLC purity was 99.7%.

Example 3

0.6 g of the crude sodium salt of pravastatin was dissolved in 5 ml of distilled water. The protocol described in Example 1 was used with the exception of the mobile phase used (30% aqueous methanol solution) and the pooled fractions with a HPLC purity of 99.8% were obtained.

Example 4

The method described in Example 3 was repeated wherein the concentration of the displacer in the mobile phase was 14%. In the fractions pooled, according to the criterion described in Example 1, HPLC purity was 99.8%.

Example 5

Pravastatin lacton (0.4g, HPLC purity 85%) was dissolved in 33 ml of the mobil phase A containing 45% methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 × 10 mm. The column was washed with the mobile phase B containing 2% of diethyleneglycoldibutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 70% methanol.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.7%.

Example 6

Pravastatin lacton (0.3g, HPLC purity 85%) was dissolved in 80 ml of the mobil phase A containing 30% methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Licrosphere RP 18 column, particle size 12 µm, column size 200 × 10 mm. The column was washed with the mobile phase B containing 5% of diethyleneglycolmono-n-hexylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance

was measured at 235 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 90% methanol. The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 7

Pravastatin lacton (0.3g, HPLC purity 85%) was dissolved in 25 ml of the mobil phase A containing 35% acetonitrile. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Licrosphere RP 18 column, particle size 12 µm, column size 200 × 10 mm. The column was washed with the mobile phase B containing 1% of diethyleneglycoldibutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 235 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 90% methanol. The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 8

The method described in Example 7 was repeated wherein the mobile phase B was 0.85% diethylphthalat in the mobile phase A.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 9

Simvastatin lacton (0.42g, HPLC purity 87%) was dissolved in 6 ml of the 66% acetonitrile and hydrolysed with 1.2mmol of sodium hydroxide. Acetonitrile was removed and pH was adjusted to 7 with diluted H_3PO_4. The column was equilibrated with mobile phase A containing 14% of methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 x 10 mm. The column was washed with the mobile phase B containing 6.7% of diethyleneglycolmono-n-hexylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 0.5ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 10

Simvastatin lacton (0.5g, HPLC purity 87 %) was dissolved in 20 ml of the mobile phase containing 70% of methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 x 10 mm. The column was washed with the mobile phase B containing 3% of decanoic acid in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 0.75 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol. The obtained fractions were analyzed by the herein above described method. The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.7%.

Example 11

Simvastatin lacton (0.5 g, HPLC purity 87%) was dissolved in 20 ml of the mobile phase containing of 60% acetonitrile. The column was equilibrated with mobile phase A . The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 x 10 mm. The column was washed with the mobile phase B containing 2% of tetrakis(decyl)amonium bromide in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 12

Lovastatin lacton (0.5g, HPLC purity 87%) was dissolved in 60 ml of the 75% methanol. The column was equilibrated with mobile phase A containing 70% of methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 x 10 mm. The column was washed with the mobile phase B containing 70% of methanol and 4.5% of decanoic acid in mobile phase A at the flow rate of 6 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.9%.

Example 13

Lovastatin lacton (0.42g, HPLC purity 87 %) was dissolved in 8 ml of the 50% acetonitrile and hydrolysed with 1.5 mmol of sodium hydroxide. Acetonitrile was removed and pH was adjusted to 7 with diluted H_3PO_4. The column was equilibrated with mobile phase A containing 14% of methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 × 10 mm. The column was washed with the mobile phase B containing 6.7% of diethyleneglycolmono-n-hexylether in mobile phase A at the flow rate of 1 ml/min. Absorbance was measured at 260 nm, and the 0.25 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The obtained fractions were analysed by the method described in example 9. The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 14

Mevastatin lacton (0.5g, HPLC purity 85%) was dissolved in 150 ml of the mobile phase A containing 70% of methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 × 10 mm. The column was washed with the mobile phase B containing 4.5% of decanoic acid in mobile phase A at the flow rate of 6 ml/min. Absorbance was measured at 260 nm, and the

1 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Claims

1. A process for obtaining HMG-CoA reductase inhibitors, characterised in that one of the steps in the process of the purification of crude HMG-CoA reductase inhibitors includes displacement chromatography which involves the use of a displacer for displacing the HMG-CoA reductase inhibitor.

2. A process according to claim 1, characterised in that the HMG-CoA reductase inhibitor is selected from the group consisting of mevastatin, pravastatin, lovastatin, simvastatin, fluvastatin and atorvastatin.

3. A process according to claim 1 or 2, characterised in that the HMG-CoA reductase inhibitor is in the lactone form or in the form of the acid or the salt thereof.

4. A process according any one of claims 1 to 3, characterised in that the displacement chromatography includes the following steps:

a) conditioning a chromatography column with a mobile phase,

b) feeding HMG-CoA reductase inhibitor dissolved in the mobile phase,

c) introducing the displacer for displacing the HMG-CoA reductase inhibitor from the column, and

d) obtaining the purified HMG-CoA reductase inhibitor.

5. A process according to claim 4, characterised in that the purified HMG-CoA reductase inhibitor is obtained by

d1) collecting the fractions, and

d2) analyzing the fractions with analytical HPLC and pooling the fractions depending on the quality of purity.

6. A process according to claim 4 or 5, characterised in that the displacement chromatography further includes the subsequent step of:
e) regenerating the chromatography column by washing the column with alcohol/water mixture to elute the displacer.

7. A process according to claim 4, characterised in that the mobile phase is selected from the group of solvents consisting of water, acetonitrile/water solutions or aqueous solutions of lower alcohols, as well as bufferred dilute solutions of organic, halogenated organic or inorganic acids with alkaline metal cations, with ammonia or with amines.

8. A process according to claim 7, characterised in that the mobile phase is any one of water, an acetonitrile/water solution or an aqueous solution of lower alcohols.

9. A process according to claim 4, characterised in that the pH of the mobile phase used is between 4.5 and 10.5.

10. A process according to claim 9, characterised in that the pH of the mobile phase used is between 6.5 and 8.

11. A process according to claim 10, characterised in that the pH of the mobile phase used is 7.

12. A process according to claim 4, characterised in that the flow rate of the mobile phase through the chromatographic column is between 1.5 and 30 ml/(min cm^2).

13. A process according to claim 4, characterised in that the flow rate of the mobile phase/displacer mixture through the chromatographic column is between 3 and 15 ml/(min cm^2).

14. A process according to claim 6, characterised in that the stationary phase is regenerated with 20 to 100% aqueous solution of lower alcohols after completed chromatography.

15. A process according to claim 4, characterised in that the stationary phase is a reverse phase.

16. A process according to claim 15, characterised in that the stationary phase is a natural reverse phase such as silica gel with alkyl chains of different lengths.

17. A process according to claim 15, characterised in that the stationary phase is either C-18 or C-8.

18. A process according to claim 15, characterised in that the stationary phase is a synthetic cross-linked polymer matrix.

19. A process according to claim 18, characterised in that the cross-linked polymer matrix is a copolymer of styrene and divinylbenzene.

20. A process according to claim 4, characterised in that the particle size of the stationary phase is between 3 and 20 µm.

21. A process according to claim 20, characterised in that the particle size of the stationary phase is between 7 and 15 µm.

22. A process according to claim 4, characterised in that the displacer is selected from the group consisting of long chain alcohols, long chain carboxylic acids, long chain alkyl ammonium salts, aromatic dicarboxylic acid

esters, oxo- and dioxo-alcohols, polyalkylene polyglycol ethers and polyaryl or polyalkylene polyaryl ethers.

23. A process according to claim 4, characterised in that the concentration of the displacer in the mobile phase is between 1 and 35%.

24. A process according to claim 23, characterised in that the concentration of the displacer in the mobile phase is between 2 and 20%.

25. The use of a process according to any one of claims 1 to 24 for producing a HMG-CoA reductase inhibitor with a HPLC purity exceeding 99.7%.

Docket No.
2260/106

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which

(check one)

- ☐ is attached hereto.
- ☒ was filed on **17 September 1999** as United States Application No. or PCT International Application Number **PCT/IB99/01553**
  and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
P 9800241	**Slovenia**	**18 September 1998**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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